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A QUALITATIVE HISTOLOGICAL STUDY IN
PRENATAL AND POSTNATAL LIVER
OF LABORATORY MICE

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Master of Arts in Biology

by
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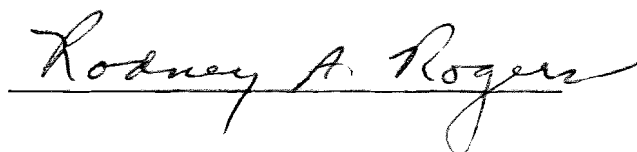
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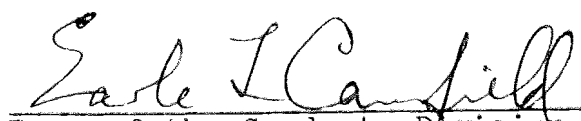
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TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. HISTORY	3
III. MATERIALS AND METHODS	9
IV. DATA	17
V. DISCUSSION	22
VI. SUMMARY AND CONCLUSIONS	27
BIBLIOGRAPHY	29
APPENDIX	32

LIST OF TABLES

TABLE	PAGE
I. The Number of Livers Used and Sections Counted Per Weekly Interval as Compared to the Eight Litters	10
II. The True Mean of the Numbers of Mouse Liver Nuclei Per mm^3 in Mice of Different Ages	18
III. The Mean Percentage of the Liver Cell Population Composed of Major Cell Types in Mice of Different Ages	21
IV. The True Numbers in Fields of Mouse Liver Nuclei at One Week Prenatal Per mm^3	33
V. The True Numbers in Fields of Mouse Liver Nuclei at Birth Per mm^3	34
VI. The True Numbers in Fields of Mouse Liver Nuclei at One Week Postnatal Per mm^3	35
VII. The True Numbers in Fields of Mouse Liver Nuclei at Two Weeks Postnatal Per mm^3	36
VIII. The True Numbers in Fields of Mouse Liver Nuclei at Three Weeks Postnatal Per mm^3	37
IX. The True Per Cent Expressing Differences in Fields of Mouse Liver Nuclei at One Week Prenatal	38
X. The True Per Cent Expressing Differences in Fields of Mouse Liver Nuclei at Birth	39
XI. The True Per Cent Expressing Differences in Fields of Mouse Liver Nuclei at One Week Postnatal	40
XII. The True Per Cent Expressing Differences in Fields of Mouse Liver Nuclei at Two Weeks Postnatal	41

LIST OF FIGURES

FIGURE		PAGE
1.	True Mean of Parenchyma and Littoral Nuclei from One Week Prenatal to Three Weeks Postnatal Per Cubic Millimeter	18
2.	True Mean of Duct, Connective, and Blood Vessel Nuclei from One Week Prenatal to Three Weeks Postnatal Per Cubic Millimeter . .	18
3.	True Percentage Mean of Parenchymal and Littoral Nuclei from One Week Prenatal to Three Weeks Postnatal	21
4.	True Percentage Mean of Duct, Connective, and Blood Vessel Nuclei from One Week Prenatal to Three Weeks Postnatal	21

CHAPTER I

INTRODUCTION

Several investigators have described postnatal growth in rodent livers. McKellar¹ found the rat parenchyma cells, sparse at birth, increased in number during the first eight weeks after birth. Daoust and Cantero² compared rat livers at birth and at sixty days. Hinson³ determined the mean number of cells per liver at various postnatal stages. Most investigators have counted nuclei in postnatal liver, but have not described changes in the relative frequency of liver cell types or their growth in prenatal livers.

It was the purpose of this investigation to describe, quantitatively, the changing cell population in the mouse liver from fourteen days after fertilization through three weeks of development after birth. The information may be

¹M. McKellar, "Postnatal Growth and Mitotic Activity of Liver of Albino Rat," American Journal of Anatomy, LXXXV (July, September, November, 1949), 292.

²Roger Daoust, "The Cell Population of Liver Tissue and the Cytological Reference Base," Liver Function, Ralph Brauer, editor (American Institute of Biological Sciences Publication, No. 4, Baltimore: Waverly Press, Inc., 1958), p. 758.

³Charles M. Hinson, "Effects of Injected Tissue Preparations on the Nuclear Growth of the Liver Cells of White Mice," (Published Master's thesis, Drake University, Des Moines, Iowa, 1964), p. 36.

of value in clarifying at what time in development cell changes occur and in describing the normal growth of hepatic cells in mice.

CHAPTER II

HISTORY

Many investigators have treated the liver as a homogeneous organ in a series of studies based on homogenates. Liver histology has been studied extensively, but cytological information is less detailed. Some recent cytological studies of the liver have been primarily concerned with the analysis of dimensions of cells and nuclei.¹ A great deal of work has been carried out on homogenates, in which nuclear counts are used to determine the number of cells in the homogenates represented.

Daoust emphasized that the liver was not the homogeneous organ that earlier investigators considered it but consisted of many different kinds of cells. He distinguished the following cellular elements: a) in the lobules; parenchymal cells and littoral cells; b) in the interlobular spaces; bile duct cells, connective tissue cells, and the cells of the blood vessel walls.²

Daoust found the true percentage of cell types in rat liver were: parenchymal (60.6), littoral (33.4), bile duct (2.0), connective tissue (2.2), and blood vessel

¹McKellar, op. cit., p. 264-265.

²Daoust, op. cit., p. 4.

walls (1.8).¹ Yokahama, Wilson, Tsuboi, and Stowell presented similar evidence in their investigations with the liver of mice.² These studies were based on apparent distribution or actual counts.

Needham claims that 44 per cent of the adult mouse liver cells are non-parenchymal and 56 per cent are parenchymal cells. This is an estimate, partly because the proportion of cell types is not constant in all parts of the liver.³

Characterization of the cell population in the liver is important as liver homogenates have been studied intensively. Differences in homogenates may arise from differing cellular composition as well as from changes in the nature of the cells present resulting from experimental conditions. Thus, the significance of the average liver cell or the average gram of liver tissue as revealed by study of homogenates depends on proportions of each kind of cell as well as the size and actual number of cells and nuclei. Growth and differentiation during development, as

¹ Ibid., p. 4.

² Hisako O. Yokahama and others, "Regeneration of Mouse Liver after Partial Hepatectomy," Cancer Research Journal, XIII (January-June, 1953), 91.

³ A. E. Needham, The Growth Process in Animals (Princeton: D. Van Nostrand Company, Inc., 1964), p. 3.

well as aging, have important effects on the liver cell population; as a result, the meaning of the average cell can be expected to change during the life cycle.

Studies were carried out on postnatal rodents and the investigators did not usually report the age of their specimens. McKellar described changes in the livers of young rats, noting the gradual decrease in hematopoiesis at seven days of age. The greatest mitotic activity was found at the constrictions of blood vessels and the zone of the lobule.¹ Wilson and Leduc determined that an increase in the number of larger nuclei parallels the development of the characteristic cell structure of the lobule.²

Jacobj studied the liver of a twenty millimeter mouse embryo and a newborn mouse. In the embryo, he found all the hepatic nuclei to be of uniform size (6.875 microns in diameter) and practically no binucleate cells were observed. In the newborn mouse, he described the majority of cells to have small nuclei (6.875 microns in diameter); but a few contained larger nuclei (8.750 microns in dia-

¹McKellar, op. cit., p. 272-278.

²J. W. Wilson and E. H. Leduc, "The Occurrence and Formation of Binucleate and Multinucleate Cells and Polyploid Nuclei in Mouse Liver," American Journal of Anatomy, LXXXIII (January, March, May, 1948), 363.

meter); the corresponding increase due to the volume doubling. He determined that while some of the smaller nuclei are still present in the adult, the 8.750 micron size was most frequent.¹ Hinson concluded this study; "There is a smaller number of nuclei per liver than that which was expected because the number of nuclei per gram is lower." "This decrease occurs because the cells are growing larger with increasing age."²

Wilson and Leduc found the number of cells per liver stabilizes at about three weeks of age. All increases in size observed after three weeks of age are the result of the development of nuclear classes and increasing cellular volume.³

A more complete study of cellular differentiation is summarized by McKellar. He stated:

The growth of liver after birth differs distinctly from its embryonic growth in which there is merely a vast system of mitotically active and relatively undifferentiated cells: for owing to the almost complete cellular differentiation which occurs after birth, mitotic activity and gross microscopic changes are limited and associated with the changes in hepatic structure, there is also a

¹Walther Jacobj, "Uber das Rhythische Wachstum der zellen Durch Verdopplung ihres Volumens," Wilhelm Roux Archiv fur Entwicklungsmechanik der Organismen, CVI (1929), 189.

²Hinson, op. cit., p. 51.

³Wilson, op. cit., p. 381.

regression of the hemopoietic properties of the liver--indeed it is not until this occurs that the liver takes on the typical microscopic appearance¹ and lobular structure of the growing adult organ.

At birth, mitotic activity occurs predominantly in the blood islands. Primarily, these are reticular cells or precursors of the von Kupffer and undifferentiated lining cells. These primitive lining cells composing the young livers are characterized by their incongruous nuclei and their isolated position between the sinusoid and hepatic cells. The bile duct cells vary in form from cuboidal to columnar and the nuclei, instead of being spherical or slightly oval, are of diverse shapes and sizes. These early hepatic cells resemble the adult cell in shape and staining properties; however, their cytoplasm is more granular.²

The liver at one week of development presents a totally different appearance. Reticular tissue (connective) has increased everywhere, but particularly about the sinusoids. With the decline in hematopoiesis, the mitotic activity of the liver increases.³

During the second week mitotic activity increases twofold producing further consolidation of the liver cells.

¹McKellar, op. cit., p. 264.

²Ibid., pp. 271-277.

³Ibid., p. 272.

This increased activity is carried into the third week, but the texture of the tissue becomes less dense by the end of this week. The reticulum has increased in the vessels and, to a lesser extent, in the bile ducts.¹

¹Ibid., pp. 273-274.

CHAPTER III

MATERIALS AND METHODS

The general plan of this investigation was to study the differentiation of hepatic cells from fourteen days after fertilization (i.e., one week prenatal) through three weeks of development after birth at weekly intervals. The mice used were from a laboratory stock maintained at Drake University. They were housed in standard mouse cages with wire gauze bottoms. Purina laboratory chow and water were available at all times. Mating was observed, and the time recorded, as the start of the prenatal period. The postnatal period commenced at the time of birth.

Eight litters of mice were used. Five livers were taken at each weekly interval and five sections per liver were studied. All sections were taken from the right lobe, lobus hepatis dexter. This lobe was selected because of its size, an important factor when using prenatal mice. Sections were taken from different areas of the lobe to give a random sampling. Table I summarizes the samples used.

The mice were killed by a sharp blow to the back of the head. No drugs or anesthetic were used as they might distort or damage the tissue. Removal of the preferred liver lobe followed immediately after death.

TABLE I

THE NUMBER OF LIVERS USED AND SECTIONS COUNTED
PER WEEKLY INTERVAL AS COMPARED
TO THE EIGHT LITTERS

WEEKLY INTERVALS	Livers and Sections Used	LITTERS							
		1	2	3	4	5	6	7	8
-1 Week	Livers	2	3						
	Sections	10	15						
Birth	Livers			1	1	3			
	Sections			5	5	15			
+1 Weeks	Livers			1	1		3		
	Sections			5	5		15		
+2 Weeks	Livers			1	1			3	
	Sections			5	5			15	
+3 Weeks	Livers			1	1				3
	Sections			5	5				15

The tissue was then placed in a fixative, Lavdowsky's Fluid:¹

Water	100 ml.
Potassium dichromate	5 g.
Mercuric chloride	0.1 g.
Glacial acetic acid	2 ml.

This fixative was chosen because the low percentage of mercuric chloride would permit the specimens to be kept for reasonably long periods of time without danger of hardening. The specimens were left in Lavdowsky's Fluid for twenty-four hours and then washed in running water overnight.

To remove the fixative and prepare the liver samples for dehydrating, Lugol's Iodine Solution² was used. The mercuric chloride in the fixative is best removed by an iodine solution.

Lugol's Iodine Solution:

Potassium iodide	1 g.
Iodine	0.5 g.
Water	50 ml.

The liver specimens were soaked for eight hours in

¹Peter Gray, Handbook of Basic Microtechnique (New York: McGraw-Hill Book Company, 1964), p. 87.

²Ibid., p. 92.

the iodine solution and then transferred to a series of ethyl alcohol baths for dehydrating. Five ethanol baths were used: 15%, 40%, 75%, 95%, and 100%. The tissues were left in each bath long enough to become impregnated with the alcohol, approximately thirty minutes in each bath. The alcohol was then removed in two baths of ninety-five per cent xylol, thus preparing the specimens for embedding.

The embedding process was performed in a home-made embedding oven. This oven consisted of a cardboard box with a cellophane window and a sixty watt light bulb as the heating source. Granular histowax paraffin was used for each of the three embedding baths. The liver specimens remained in the first two baths of molten wax approximately two hours each, or until complete impregnation occurred. The third bath of molten wax was prepared in a one-half inch square paper box. After placing the specimens in the third bath, one liver per box, hardening was hastened by immersing in cool water. The specimen blocks were sectioned from one to four weeks after embedding.

An American Optical Company rotary microtome was used for sectioning. The sections, ten microns in thickness, were taken at random. Five sections per paraffin block were used in the project count.

The slices were mounted on standard three by one

inch slides, 1.2 millimeters thick. The slides were covered with Mayer's Albumen Adhesive¹ for securing the sections.

Mayer's Albumen Adhesive:

Fresh egg white	50 ml.
Glycerin	50 ml.
Sodium salicylate	1 g.

After the sections were mounted onto the slides, they were placed in a series of xylene and ethyl alcohol baths to prepare them for staining. The bath series consisted of two one hundred per cent xylene baths, a fifty per cent xylene and fifty per cent alcohol bath, and one bath of absolute alcohol. This series was for removal of the paraffin from the sections.

To prepare the slide specimens for staining, they were washed in a water bath until all the alcohol had been removed. They were then placed in Delafield's Alum Hematoxylin stain² for about five minutes or until the sections were dark purple.

Delafield's Alum Hematoxylin:

Water	70 ml.
Glycerin	15 ml.
Methanol	15 ml.

¹Gray, op. cit., p. 173.

²Ibid., p. 102.

95% ethyl alcohol	4 ml.
Ammonium alum	3 g.
Hematoxylin	0.6 g.

Upon removal of the slides from the stain, they were rinsed in distilled water and differentiated until only the nuclei remained colored. The differentiating solution consisted of a one-tenth per cent hydrochloric acid in seventy per cent alcohol mixture.¹ Differentiating was followed by dipping the slides in a bluing solution until the nuclei became a dark blue. The bluing solution was one-tenth per cent sodium bicarbonate in tap water solution.² Bluing was followed by dehydrating in an alcohol series and clearing in xylene.

The slides were then mounted with number one cover slips. These slips were fastened with a mixture of sixty grams Harleco Synthetic Resin mounting medium dissolved in forty grams of xylene.

After the mounting medium had dried and the slides were cleaned, determination of the different cells began. Five types of cells were counted:

1. Parenchyma cells, polygonal in shape, with large round, and smooth nucleus containing one or more nucleoli;

¹Ibid., p. 103.

²Ibid.

2. Littoral cells, including Kupffer cells and undifferentiated cells which cannot be easily distinguished one from the other;
3. Bile duct cells, cuboidal cells, with only one elongated nucleus found per cell;
4. Blood vessel cells, cells with an elongated and flattened nucleus, sometimes curved with the lumen of the vessel, nucleus lacks nucleoli but contains disk-like chromatin particles;
5. Connective tissue, cells columnar, cuboidal or squamous, nucleus elongated with simple shape and one or more nucleoli.¹

A standard American Optical Microscope was used for counting the cells. An oil immersion objective of one hundred magnifications was used in combination with an eyepiece of ten magnifications.

A Whipple eyepiece formed the grid. All cells whose nuclei were included in the grid were tabulated. The total number of cells, as evidenced by the nuclei, were recorded, and the numbers of cells falling into the five categories listed above were also recorded.

Counts were made for each of one hundred and twenty-five

¹William Bloom and Don W. Fawcett, Textbook of Histology (Philadelphia: W. B. Saunders Company, 1962), p. 462-478.

liver sections. One grid was counted per section. The site of counting was selected randomly.

The mean nuclear sizes were calculated from measurements, by ocular micrometry, on 20 parenchymal cells, 20 littoral cells, and 5 cells of the other three types for each weekly stage. This data was corrected for relative nuclear sizes as outlined by Abercrombie's equation:

$$P = A \frac{M}{L + M}$$

P is the correct number of nuclei, A is the apparent number of nuclei, L is the nuclear diameter, and M is the section thickness.¹

The total volume of the microscopic field was calculated in cubic millimeters using the measurements of 10 microns, thickness; 180.2 microns, radius; and 3.14, pi. The total volume of the areas counted in each section was 5658.3u³, and to correct to a value representing lmm³, a factor of 980.1 was required.

¹M. Abercrombie, "Estimation of Nuclear Population from Microtome Sections," Anatomical Record, XCIV (1946), 240.

CHAPTER IV

DATA

The number of nuclei of each cell type per mm^3 was determined for each field selected as a sample; these results are reported in Tables IV-VIII. These results are summarized in Table II, in which the true mean of mouse liver nuclei for each cell type at each week is shown. The means are also shown in graphic form in Figures 1 and 2.

TABLE II

THE TRUE MEAN OF THE NUMBERS OF
MOUSE LIVER NUCLEI PER mm^3 IN
MICE OF DIFFERENT AGES

Field Mean	Paren. 10^4	Litt. 10^4	Duct 10^3	Conn. 10^2	B. Ves. 10^3	Total 10^4
-1 wk.	8.6	21.2	1.2	0.0	0.1	30.1
Birth	10.2	10.8	3.6	0.9	4.5	22.0
1 wk.	10.8	5.0	6.7	2.0	4.0	17.0
2 wks.	13.8	2.6	5.1	34.9	8.9	18.1
3 wks.	11.0	0.9	4.6	3.1	5.0	13.2

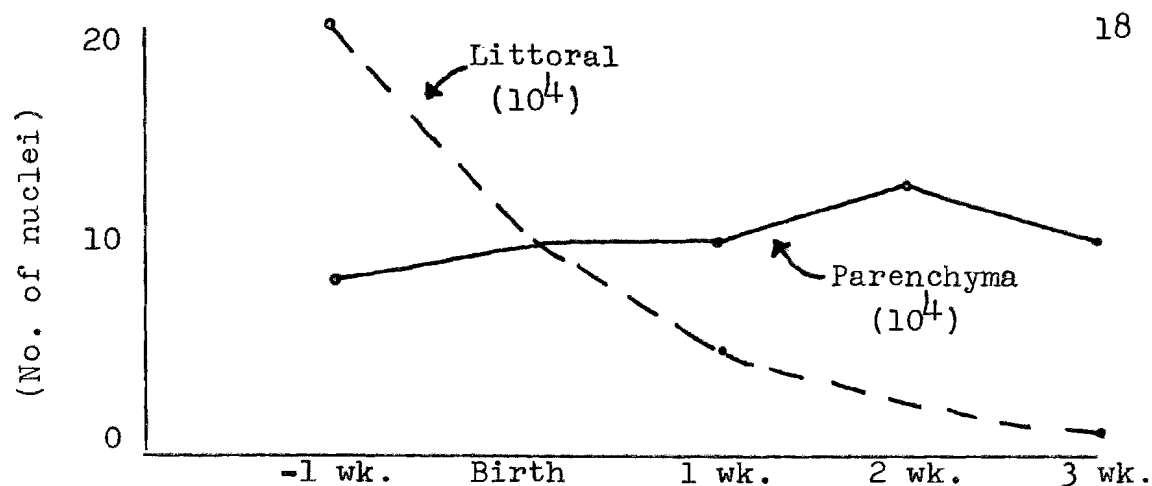


Figure 1. True mean of parenchyma and littoral nuclei from one week prenatal to three weeks postnatal per cubic millimeter.

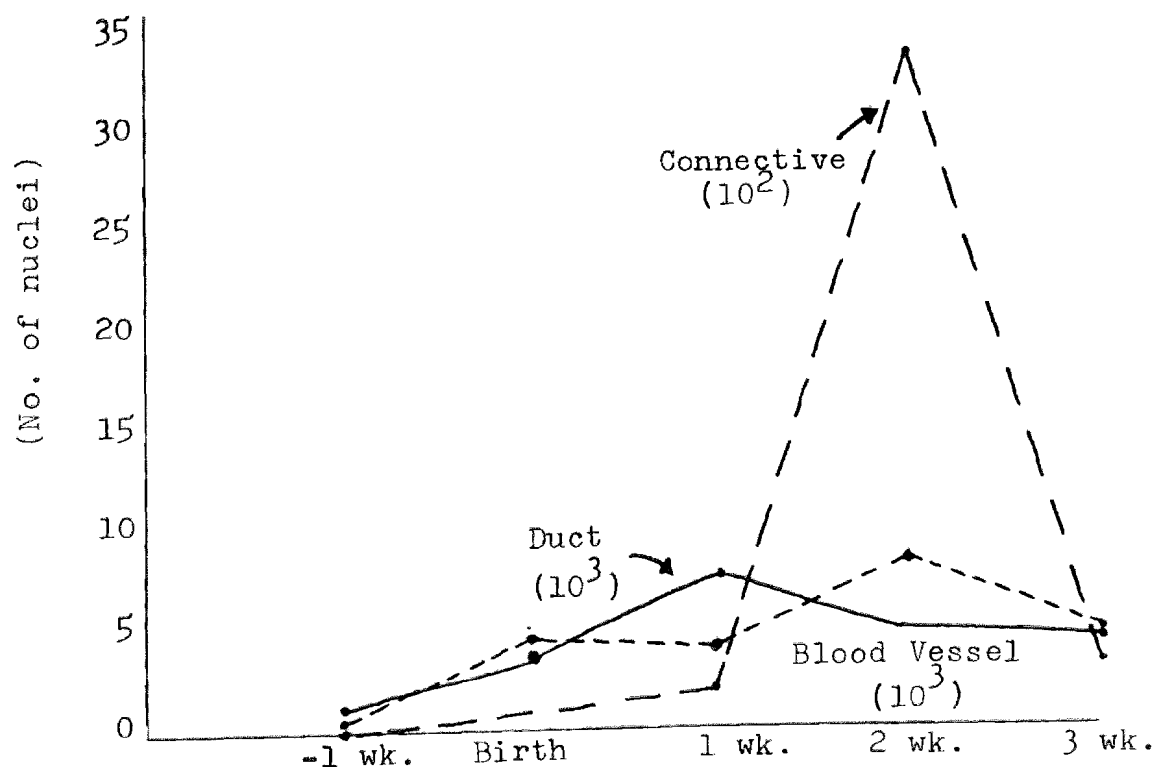


Figure 2. True mean of duct, connective, and blood vessel nuclei from one week prenatal to three weeks postnatal per cubic millimeter.

All cell types increased in number with the exception of the littoral cells. The increased number of most cell types undoubtedly reflects changes in the cell population associated with cell differentiation and multiplication as the liver differentiates and assumes a histological structure like that of adult liver. The sharp decrease in littoral cells, defined in this study as cells that were not determinable, also, reflects cellular differentiation and the decline in hematopoietic activity.

The percentage of the population of nuclei categorized as parenchymal, littoral, duct, connective, and blood vessel was determined, using the true numbers after all correction factors had been applied. The percentages obtained for each field studied are shown in Tables IX-XIV. The data is summarized in Table III and Figures 3 and 4.

The percentage of the cell population made up of littoral cells decreased, the other four cell types increased. The changes were similar in direction, but different in proportion, to the changes in the number of the various cell types per mm^3 of liver tissue.

TABLE III

THE MEAN PERCENTAGE OF THE LIVER CELL
POPULATION COMPOSED OF MAJOR CELL
TYPES IN MICE OF DIFFERENT AGES

Age	Parenchymal	Littoral	Duct	Connective	Blood Vessel
-1 wk.	29.0	70.5	0.4	0.0	0.0
Birth	46.6	49.1	1.7	0.4	2.2
1 wk.	64.0	29.1	4.3	0.1	2.5
2 wks.	76.2	14.3	2.8	1.9	4.8
3 wks.	85.8	6.5	3.6	0.3	3.8

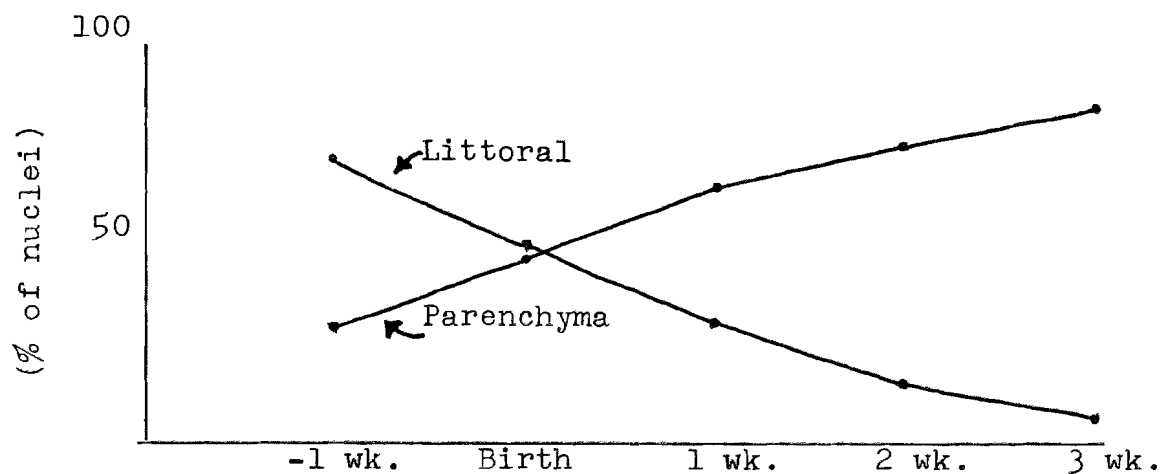


Figure 3. True percentage mean of parenchymal and littoral nuclei from one week prenatal to three weeks postnatal.

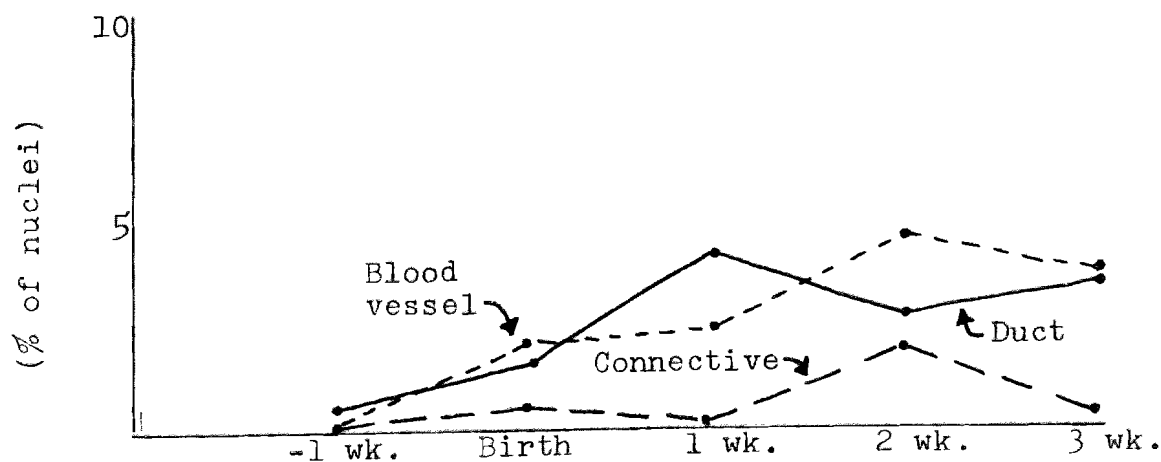


Figure 4. True percentage mean of duct, connective, and blood vessel nuclei from one week prenatal to three weeks postnatal.

CHAPTER V

DISCUSSION

The appearance and shape of liver cells change drastically during development. Most investigators have studied the adult liver and found it to be composed primarily of parenchyma cells. Daoust, McKellar, Yokahama, Needham, and others have found parenchymal cells making up from 50 to 60 per cent of the rodent liver cell population, with non-parenchymal cells composing the remaining 40 to 50 per cent.

An important event during liver development is the decrease and eventual cessation of hematopoiesis. As the hematopoietic cells mature and move into the blood stream without being completely replaced, a definite decrease in the total number of liver cells per mm³ is noticed. The increase in average cell volume is undoubtedly, in part, a result of the depletion of these tiny cells. It should be noted that the hematopoietic cells were included in the littoral cell category in this study. There can be no doubt that the decrease in number and percentage of littoral cells is partly a result of the decline in hematopoietic activity. As the hematopoietic cells migrate into the blood stream, all other liver components become more identifiable. This occurred gradually and was, for the

most part, completed by the end of one week postnatal. The number and percentage of liver cells continued to decline after this, but not as rapidly as in younger mice.

As lobular structure becomes more apparent the connective tissue lining the lobes thickens and is more visible than in younger mice. McKellar emphasized the emergence of normal lobular structure during the first few weeks after birth. As this occurs, the connective tissues associated with the periphery of the lobule might be expected to appear and increase, as is seen in Figures 2 and 4. But, for an organ of its size, the liver has remarkably little connective tissue which increases slowly throughout life.¹

As the liver assumes a histological structure more like that of the adult, the duct and parenchymal cells become prominent. The formation of the bile duct system requires more cells as the system increases in size. Therefore, a more gradual increase in duct cells than was observed (see Figures 2 and 4) might be expected. The number of duct cells per mm³ decreased after the first postnatal week and the percentage of the cell population composed of duct cells appears to have stabilized after

¹William Bloom and Don W. Fawcett, A Textbook of Histology (Philadelphia: W. B. Saunders Co., 1968), p. 598.

this time. It is not clear whether sampling errors are responsible, or the budding of duct cells to form parenchyma is reflected in the data. If, as Wilson and Leduc report, the cell population has become stabilized by three weeks after birth, the data may reflect the end of the budding process and the achievement of a stabilized percentage of duct cells.

Parenchymal increase was continual throughout development. Enlargement of average cell size is an important indication of a maturing liver. Growth of average cell size is undoubtedly a result, in part, of the decrease of littoral (and hematopoietic) cells and increase in size and number of parenchymal cells. Cell enlargement resulted from cytoplasmic growth, which may be associated with increased nuclear volume resulting from an increase in average ploidy of parenchymal cell nuclei. The increase in size of parenchymal cells is clearly reflected in the data. During the last three weeks, no significant increase in the number of parenchymal cell nuclei per mm^3 was observed, although the percentage of the cell population made up of parenchymal cells was constantly on the rise.

In this investigation, littoral cells were considered those that could not be identified as members of other categories, or were hematopoietic. The result was that

the cells lining the sinusoids were included with the parenchymal cells. The percentage of parenchymal cells reported here is, therefore, equivalent to parenchymal and littoral cells in the reports of Daoust and Needham, and the resulting percentages are very nearly the same. As cells lining the sinusoids do not enlarge as much as parenchymal cells, the actual enlargement of the parenchymal cells is minimized in the relationship of percentages and numbers of nuclei per mm^3 in this study.

The parenchymal cells, as was expected, increased greatly during the early stages of development, not only in per cent of cell population, but in nuclear size. The littoral cells, nondeterminant cells, decreased in per cent of cell population and were fewer per mm^3 . The parenchymal rise was almost inversely proportional to the decrease in littoral cells. This would indicate that parenchymal cells arise from differentiating littoral cells as well as from mitosis of parenchymal cells and budding of the bile duct.

The percentage of cell types described here are different than those reported by Daoust and Cantero for the newborn rat. They described 61 per cent to be parenchymal cells, 30 per cent littoral cells, 4.0 per cent duct cells, 2.2 per cent connective cells, and 2.7 per cent

blood vessel wall cells.¹ This investigator found these percentages: 46.6 per cent parenchymal, 49.1 per cent littoral, 1.7 per cent duct, 0.4 per cent connective, and 2.2 per cent blood vessel wall cells at birth in the mouse.

Part of the discrepancy may result from the use of mice in the present study and rats by Daoust and Cantero; the major difference is undoubtedly the result of a different definition of littoral cells in the two studies.

Daoust considered littoral cells as being indeterminant cells and Von Kupffer cells. This investigator considered them only as indeterminant cells composed primarily of hematopoietic cells. This investigation used fixed and stained livers while Daoust, primarily, used fresh homogenate livers.

Normally, embryology is studied through organ development; however, there is an advantage of investigating this field through quantitative histology. The shifting cell populations, as revealed by the changing number of cells per unit of organ volume, and the changing proportion of cell types, provide valuable information that can be obtained in no other way. In this study, the changing numbers and percentages of cell types was found to correlate well with the major events in liver differentiation.

¹Roger Daoust and A. Cantero, "The Numerical Proportions of Cell Types in Rat Liver During Carcinogenesis by 4-Dimethylaminoazobenzene (DAB)," Cancer Research, XIX (1959), 758.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to describe the differentiation of mouse hepatic cells from fourteen days after fertilization through three weeks of development after birth. Eight litters of mice were used. Five livers were taken at each weekly interval and five sections per liver studied. All sections were taken from the right lobe, lobus hepatis dexter. Counts of each cell type--parenchyma, littoral, duct, connective tissue, and blood vessel cells--were taken for each of the one hundred and twenty-five liver sections. The site of counting was selected at random. The true number of cells per cubic millimeter and percentage of each cell type was calculated.

The number of parenchymal cell, bile duct cell, connective tissue cell, and blood vessel cell nuclei per mm^3 increased steadily, but not in a straight line relationship, throughout the period of study, while the number of littoral cell nuclei decreased. Changes in the numbers of nuclei per mm^3 and in the percentage of the cell population composed of the various categories of cells correlated well with changes in cell size and the major changes in liver form known to occur during the period of development studied.

Quantitative studies of this kind promise to permit a more accurate description of the rate of change in some aspects of organ differentiation.

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APPENDIX

TABLE IV

THE TRUE NUMBERS IN FIELDS OF MOUSE LIVER
NUCLEI AT ONE WEEK PRENATAL PER mm^3

Age	Paren. 10^4	Litt. 10^4	Duct 10^3	Conn. 10^2	B. Ves. 10^3	Total 10^4
-1 wk.	8.9	25.5	1.1	0.0	0.0	34.5
	6.8	29.4	0.6	0.0	0.6	36.3
	9.4	27.7	2.7	0.0	0.0	37.4
	6.7	26.5	1.7	0.0	0.0	33.4
	7.0	15.6	1.7	0.0	0.0	22.8
-1 wk.	4.8	24.0	0.6	0.0	0.0	28.9
	7.5	22.8	0.6	0.0	1.1	30.5
	8.4	20.9	0.6	0.0	0.0	29.4
	7.9	19.3	0.6	0.0	0.0	27.3
	9.4	21.1	0.0	0.0	0.0	30.5
-1 wk.	9.8	24.0	1.1	0.0	0.0	33.9
	9.8	18.6	0.0	0.0	0.0	28.4
	7.3	21.1	0.0	0.0	0.0	28.4
	8.1	19.6	1.1	0.0	0.0	27.9
	7.6	21.5	0.6	0.0	0.0	29.2
-1 wk.	8.8	19.1	1.7	0.0	0.6	28.1
	12.5	20.3	0.6	0.0	0.0	32.9
	7.1	13.8	2.1	0.0	0.0	21.1
	11.9	21.5	1.7	0.0	0.0	33.6
	8.9	19.5	2.7	0.0	0.0	28.7
-1 wk.	8.2	22.5	0.6	0.0	0.0	30.8
	12.1	23.7	0.6	0.0	0.0	35.9
	6.9	23.2	4.3	0.0	0.0	30.5
	10.1	12.5	2.7	0.0	0.0	25.3
	8.9	16.6	1.1	0.0	0.0	25.6
Total	214.8	530.3	31.1	0.0	2.3	751.3

TABLE V
THE TRUE NUMBERS IN FIELDS OF MOUSE
LIVER NUCLEI AT BIRTH PER mm³

Age	Paren. 10 ⁴	Litt. 10 ⁴	Duct 10 ³	Conn. 10 ²	B. Ves. 10 ³	Total 10 ⁴
Birth	14.5	11.9	5.4	10.8	2.8	29.9
	10.2	11.4	3.8	0.0	2.3	22.2
	9.8	12.9	2.7	0.0	1.1	23.1
	11.6	11.9	0.6	0.0	1.7	23.7
	12.9	7.2	4.3	0.0	0.0	20.5
Birth	6.7	14.2	2.7	0.0	3.3	21.5
	10.1	10.5	0.0	0.0	2.8	20.9
	10.7	10.5	2.7	0.0	3.3	21.8
	10.3	10.0	4.3	0.0	4.5	21.2
	9.0	9.7	3.2	5.9	4.5	19.5
Birth	14.3	7.5	13.0	0.0	4.5	23.6
	9.4	9.9	14.6	39.2	15.8	22.7
	9.1	7.1	3.2	10.8	9.6	17.6
	10.1	8.7	4.9	16.7	6.2	20.1
	10.4	13.9	5.4	10.8	4.5	25.4
Birth	6.7	5.9	7.6	10.8	8.4	14.3
	11.9	10.0	1.7	28.4	6.2	24.7
	10.4	13.4	2.2	16.7	5.1	24.7
	9.3	8.2	1.1	28.4	10.1	18.9
	9.7	12.2	1.1	28.4	2.8	22.6
Birth	8.8	13.9	2.2	0.0	2.8	23.2
	6.4	10.2	1.1	0.0	2.8	17.0
	8.1	9.2	1.1	16.7	3.9	18.0
	11.0	14.0	1.7	0.0	2.3	25.4
	13.5	15.1	0.0	0.0	2.3	28.8
Total	254.9	269.4	90.6	224.0	113.4	551.3

TABLE VI

THE TRUE NUMBERS IN FIELDS OF MOUSE LIVER
NUCLEI AT ONE WEEK POSTNATAL PER mm³

Age	Paren. 10 ⁴	Litt. 10 ⁴	Duct 10 ³	Conn. 10 ²	B. Ves. 10 ³	Total 10 ⁴
1 wk.	11.0	10.3	5.4	10.8	3.3	22.3
	12.8	5.4	12.0	0.0	2.8	19.7
	11.4	4.2	4.3	0.0	3.3	16.4
	11.9	3.2	6.5	0.0	3.9	16.1
	14.7	4.8	4.9	0.0	5.6	20.6
1 wk.	11.3	6.6	11.4	10.8	3.9	19.5
	14.1	7.8	4.3	0.0	3.3	22.7
	10.4	6.4	1.1	0.0	5.6	17.5
	13.6	4.3	41.7	0.0	1.7	22.2
	12.3	6.0	4.9	0.0	6.8	19.5
1 wk.	9.6	4.9	4.9	0.0	1.7	15.2
	8.5	2.6	6.0	0.0	2.3	11.9
	11.1	6.6	6.5	0.0	0.6	18.4
	11.0	4.7	11.4	16.7	2.8	17.3
	11.0	4.4	9.2	0.0	3.9	16.7
1 wk.	8.9	4.5	4.3	0.0	2.8	14.1
	9.1	2.9	4.3	0.0	5.6	13.0
	9.1	2.9	3.8	0.0	6.2	13.0
	9.0	4.4	9.2	10.8	3.9	14.8
	11.8	2.5	5.4	0.0	3.9	15.2
1 wk.	7.6	5.7	6.5	0.0	6.2	14.6
	9.2	5.5	2.7	0.0	6.8	15.7
	10.9	4.7	12.0	0.0	3.9	17.2
	9.9	5.1	3.2	0.0	3.3	15.7
	9.9	4.6	4.3	0.0	6.8	15.6
Total	270.1	125.0	190.2	49.1	100.9	424.9

TABLE VII

THE TRUE NUMBERS IN FIELDS OF MOUSE LIVER
NUCLEI AT TWO WEEKS POSTNATAL PER mm^3

Age	Paren. 10^4	Litt. 10^4	Duct 10^3	Conn. 10^2	B. Ves. 10^3	Total 10^4
2 wks.	21.2	1.1	8.6	67.6	3.3	24.2
	11.3	2.9	9.2	146.0	11.9	17.8
	20.0	1.4	4.3	72.5	10.7	23.6
	14.7	3.0	7.1	140.1	15.2	21.3
	13.5	3.1	0.0	28.4	12.9	18.2
2 wks.	13.0	3.6	6.5	61.7	19.7	19.8
	13.1	2.2	3.2	45.1	18.0	17.9
	16.1	4.1	2.7	111.7	20.9	23.7
	15.3	2.9	1.7	55.9	11.3	20.1
	16.4	2.9	0.0	28.4	10.7	20.7
2 wks.	12.2	2.7	0.0	28.4	9.0	16.1
	12.5	1.6	5.4	22.5	14.1	16.3
	14.6	1.3	15.7	39.2	17.4	19.6
	13.0	1.5	12.0	0.0	3.3	16.0
	16.7	1.9	12.0	22.5	2.8	20.3
2 wks.	12.3	0.4	6.0	5.9	3.3	13.7
	10.8	3.4	3.2	28.4	14.6	16.3
	10.3	2.2	0.6	0.0	2.8	12.8
	11.2	3.2	4.9	10.8	10.7	16.1
	13.1	2.4	6.0	0.0	3.3	16.4
2 wks.	12.6	3.0	6.5	10.8	1.7	16.5
	9.9	2.3	2.7	0.0	1.7	12.6
	11.2	2.4	2.7	0.0	0.0	13.9
	13.1	3.1	2.7	0.0	1.1	16.6
	15.8	5.4	2.7	10.8	3.3	21.9
Total	343.9	64.0	126.4	871.4	223.7	452.4

TABLE VIII

THE TRUE NUMBERS IN FIELDS OF MOUSE LIVER
NUCLEI AT THREE WEEKS POSTNATAL PER mm³

Age	Paren. 10 ⁴	Litt. 10 ⁴	Duct 10 ³	Conn. 10 ²	B. Ves. 10 ³	Total 10 ⁴
3 wks.	11.4	0.1	8.1	0.0	6.2	12.9
	8.2	0.5	4.3	16.7	11.9	10.5
	8.7	0.6	6.5	10.8	2.8	10.3
	8.6	0.6	8.1	0.0	5.6	10.6
	10.0	0.6	3.8	5.9	3.9	11.4
3 wks.	11.1	1.5	2.7	5.9	2.8	13.2
	12.5	0.7	4.9	0.0	3.3	14.0
	10.5	2.0	7.6	0.0	15.8	14.8
	14.7	1.6	6.0	0.0	6.8	17.6
	11.3	1.5	5.4	10.8	5.6	14.0
3 wks.	10.6	1.5	3.8	0.0	3.3	12.8
	11.9	2.0	4.9	22.5	3.3	14.9
	20.5	0.8	5.4	0.0	7.6	22.6
	25.7	1.2	8.6	0.0	7.4	28.5
	9.6	0.3	1.1	0.0	2.3	10.2
3 wks.	10.8	1.1	3.8	0.0	10.8	23.4
	10.1	0.6	4.9	5.9	1.7	11.4
	8.0	0.4	4.3	0.0	2.8	9.1
	7.9	0.2	1.7	0.0	5.6	8.8
	9.7	0.5	4.9	0.0	3.3	11.0
3 wks.	7.9	0.3	3.8	0.0	2.8	8.9
	9.6	0.4	2.2	0.0	2.3	10.5
	9.3	1.5	4.3	0.0	3.3	11.6
	7.9	0.5	1.1	0.0	1.7	8.7
	8.0	0.5	2.7	0.0	1.7	8.9
Total	274.5	21.5	114.9	78.5	124.6	330.6

TABLE IX
THE TRUE PER CENT EXPRESSING DIFFERENCES
IN FIELDS OF MOUSE LIVER NUCLEI
AT ONE WEEK PRENATAL

Age	Parenchyma	Littoral	Duct	Connective	Blood Vessel
-1 wk.	25.3	74.4	0.3	0.0	0.0
	18.8	80.9	0.1	0.0	0.2
	25.2	74.1	0.7	0.0	0.0
	20.2	79.3	0.5	0.0	0.0
	30.8	68.5	0.7	0.0	0.0
-1 wk.	16.7	83.6	0.2	0.0	0.0
	24.6	74.8	0.2	0.0	0.4
	28.6	71.2	0.2	0.0	0.0
	29.0	70.8	0.2	0.0	0.0
	30.7	69.3	0.0	0.0	0.0
-1 wk.	29.0	70.7	0.3	0.0	0.0
	34.4	65.6	0.0	0.0	0.0
	25.8	74.2	0.0	0.0	0.0
	29.2	70.4	0.4	0.0	0.0
	26.0	73.8	0.2	0.0	0.0
-1 wk.	31.4	67.8	0.6	0.0	0.2
	38.1	61.8	0.2	0.0	0.0
	33.5	65.5	1.0	0.0	0.0
	35.5	64.0	0.5	0.0	0.0
	31.0	68.0	0.9	0.0	0.0
-1 wk.	26.6	73.2	0.2	0.0	0.0
	33.7	66.1	0.2	0.0	0.0
	22.6	75.9	1.4	0.0	0.0
	44.2	54.6	1.2	0.0	0.0
	34.6	65.0	0.4	0.0	0.0

TABLE X
THE TRUE PER CENT EXPRESSING DIFFERENCES
IN FIELDS OF MOUSE LIVER
NUCLEI AT BIRTH

Age	Parenchyma	Littoral	Duct	Connective	Blood Vessel
Birth	53.0	43.6	1.0	0.4	1.0
	46.0	51.3	1.7	0.0	1.0
	42.3	56.1	1.2	0.0	0.5
	48.9	50.1	0.2	0.0	0.7
	62.7	35.2	2.1	0.0	0.0
Birth	31.1	66.0	1.3	0.0	1.6
	48.3	50.4	0.0	0.0	1.3
	49.1	48.1	1.2	0.0	1.5
	48.6	47.3	2.0	0.0	2.1
	46.1	49.6	1.7	0.3	2.3
Birth	60.6	31.9	5.5	0.0	1.9
	41.3	43.5	6.5	1.7	7.0
	51.6	40.5	1.8	0.6	5.5
	50.5	43.2	2.4	0.8	3.1
	40.9	54.7	2.1	0.4	1.8
Birth	47.0	41.0	5.3	0.8	5.9
	51.7	43.6	0.7	1.2	2.7
	42.1	54.3	0.9	0.7	2.1
	49.1	43.5	0.6	1.5	5.4
	43.1	54.0	0.5	1.2	1.3
Birth	38.0	59.9	0.9	0.0	1.2
	37.8	59.9	0.6	0.0	1.7
	45.0	51.3	0.6	0.9	2.2
	43.4	55.1	0.6	0.0	0.9
	46.8	52.4	0.0	0.0	0.8

TABLE XI

THE TRUE PER CENT EXPRESSING DIFFERENCES
IN FIELDS OF MOUSE LIVER NUCLEI
AT ONE WEEK POSTNATAL

Age	Parenchyma	Littoral	Duct	Connective	Blood Vessel
1 wk.	49.3	46.2	2.4	0.5	1.5
	65.1	27.4	6.1	0.0	1.4
	69.6	25.7	2.6	0.0	2.1
	73.7	19.8	4.0	0.0	2.4
	71.5	23.4	2.4	0.0	2.7
1 wk.	57.9	33.7	5.8	0.6	2.0
	62.2	34.4	1.9	0.0	1.5
	59.7	36.5	0.6	0.0	3.2
	61.3	19.2	18.8	0.0	0.8
	63.1	31.0	2.5	0.0	3.5
1 wk.	63.1	32.6	3.2	0.0	1.1
	71.1	22.0	5.0	0.0	1.9
	60.4	35.8	3.5	0.0	0.3
	63.6	27.2	6.6	1.0	1.6
	65.9	26.2	5.5	0.0	2.4
1 wk.	63.2	31.8	3.1	0.0	2.0
	70.3	22.0	3.3	0.0	4.3
	70.1	22.3	2.9	0.0	4.7
	60.7	29.6	6.2	0.8	2.5
	77.3	16.5	3.6	0.0	2.9
1 wk.	52.0	39.2	4.5	0.0	4.3
	58.7	35.2	1.7	0.0	4.3
	63.4	27.4	6.9	0.0	2.3
	63.3	32.4	2.1	0.0	2.2
	63.3	29.6	2.8	0.0	4.3

TABLE XII

THE TRUE PER CENT EXPRESSING DIFFERENCES
IN FIELDS OF MOUSE LIVER NUCLEI
AT TWO WEEKS POSTNATAL

Age	Parenchyma	Littoral	Duct	Connective	Blood Vessel
2 wks.	87.6	4.6	3.6	2.8	1.4
	63.7	16.4	5.2	8.2	6.6
	84.6	5.9	1.8	3.1	4.5
	68.8	14.2	3.3	6.6	7.1
	74.1	17.2	0.0	1.5	7.1
2 wks.	65.6	18.1	3.3	3.1	9.9
	73.3	12.3	1.8	2.5	10.1
	68.1	17.3	1.1	4.7	8.8
	76.3	14.5	0.8	2.8	5.6
	79.6	13.9	0.0	1.4	5.2
2 wks.	76.0	16.7	0.0	1.7	5.6
	76.9	9.8	3.3	1.4	8.7
	74.5	6.6	8.0	2.0	8.9
	81.1	9.4	7.4	0.0	2.1
	82.2	9.4	5.9	1.1	1.4
2 wks.	89.9	2.9	4.3	0.4	2.5
	66.3	21.0	2.0	1.7	9.0
	80.3	17.0	0.4	0.0	2.2
	69.6	20.1	3.0	0.7	6.6
	80.0	14.3	3.6	0.0	2.1
2 wks.	76.1	18.3	3.9	0.7	1.0
	78.4	18.1	2.1	0.0	1.3
	81.0	17.0	2.0	0.0	0.0
	78.8	18.9	1.6	0.0	0.7
	72.0	24.8	1.2	0.5	1.5

TABLE XIII

THE TRUE PER CENT EXPRESSING DIFFERENCES
IN FIELDS OF MOUSE LIVER NUCLEI
AT THREE WEEKS POSTNATAL

Age	Parenchyma	Littoral	Duct	Connective	Blood Vessel
3 wks.	88.4	0.4	6.3	0.0	4.8
	78.5	4.3	4.2	1.6	11.4
	84.0	5.9	6.3	1.1	2.7
	81.7	5.3	7.7	0.0	5.3
	87.9	4.9	3.3	0.5	3.4
3 wks.	83.9	11.4	2.0	0.4	2.1
	89.0	5.2	3.5	0.0	2.4
	70.8	13.5	5.1	0.0	10.6
	83.5	9.2	3.4	0.0	3.8
	80.6	10.8	3.9	0.8	4.0
3 wks.	83.0	11.4	3.0	0.0	2.6
	79.8	13.2	3.3	1.5	2.3
	93.5	3.8	2.5	0.0	0.3
	90.3	4.1	3.0	0.0	2.6
	93.4	3.3	1.1	0.0	2.2
3 wks.	80.8	8.4	2.8	0.0	8.0
	88.4	5.4	4.2	0.5	1.5
	87.8	4.3	4.8	0.0	3.1
	89.8	1.9	1.9	0.0	6.4
	87.9	4.6	4.4	0.0	3.1
3 wks.	88.8	3.8	4.3	0.0	3.2
	92.0	3.8	2.1	0.0	2.2
	80.6	12.7	3.8	0.0	2.9
	91.0	5.8	1.2	0.0	1.9
	89.4	5.7	3.0	0.0	1.9

TABLE XIV

43

THE TRUE PERCENTAGE MEAN OF MOUSE LIVER
NUCLEI FROM ONE WEEK PRENATAL
TO THREE WEEKS POSTNATAL

Field Ave.	Parenchyma	Littoral	Duct	Connective	Blood Vessel
-1 wk.	24.0	75.4	0.5	0.0	0.0
	25.9	73.9	0.1	0.0	0.7
	28.9	71.0	0.2	0.0	0.0
	33.9	65.4	0.6	0.0	0.4
	32.4	67.0	0.7	0.0	0.0
Mean	29.0	70.5	0.4	0.0	0.0
Birth	50.6	47.3	1.4	0.1	0.7
	44.0	52.3	1.2	0.1	1.8
	49.0	42.8	3.7	0.7	3.8
	46.6	47.3	1.6	1.1	3.5
	42.2	55.5	0.6	0.2	1.3
Mean	46.6	49.1	1.7	0.4	2.2
1 wk.	65.9	28.5	3.5	0.1	2.0
	60.8	31.0	5.9	0.1	2.2
	64.8	28.8	4.8	0.2	1.5
	68.3	24.4	3.8	0.2	3.3
	60.2	32.8	3.6	0.0	3.5
Mean	64.0	29.1	4.3	0.1	2.5
2 Wks.	75.8	11.7	2.8	4.4	5.4
	72.6	15.2	1.4	2.9	7.9
	78.1	10.4	4.9	1.2	5.3
	77.2	15.1	2.7	0.6	4.5
	77.3	19.4	2.2	0.2	0.9
Mean	76.2	14.3	2.8	1.9	4.8
3 Wks.	84.1	4.2	5.5	0.6	5.5
	81.6	10.0	3.6	0.2	4.6
	88.0	7.4	2.6	0.3	2.0
	86.9	4.9	3.6	0.1	4.4
	88.4	6.3	2.9	0.0	2.4
Mean	85.8	6.5	3.6	0.3	3.8